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Research article

Kernel size and weight affected by three plant bioregulators applied at bloom to Non Pareil and Carmel almond cultivars

Segundo Maita *[,1,2], Carlos Sotomayor [1]

[1] Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile
[2] Facultad de Ciencias Agropecuarias, Universidad de Cuenca, Cuenca, Ecuador

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ABSTRACT

Background: The yield of almonds [*Prunus dulcis* (Mill.) D.A. Webb] could be low due to climatic problems and any factor improving kernel size and weight, such as the use of plant bioregulators (PBRs), should be beneficial.
Results: Three plant bioregulators: 24-epibrassinolide (BL), gibberellic acid (GA_3) and kinetin (KN) were applied at three spray concentrations to Non Pareil and Carmel cultivars, at two phenological stages during bloom, in the 2014 and 2015 seasons. The results showed significant differences ($P < 0.0001$). For total dry weight of Non Pareil, the best treatment was BL (30 mg·L^{-1}), with an average of 1.45 g, while the control was 1.30 g, at pink button during 2015. For Carmel, the best dry weight was 1.23 g, achieved with BL (30 mg·L^{-1}) at fallen petals in both seasons. The average dry weight of the controls varied between 1.13 and 1.18 g. The greatest almond lengths and widths in Non Pareil were 24.98 mm and 15.05 mm, achieved with BL (30 mg·L^{-1}) and KN (50 μL·L^{-1}) treatments, respectively, applied at pink button in 2015. In Carmel, the greatest length and width were 24.38 and 13.44 mm, obtained with BL (30 mg·L^{-1}) applied at the stages of pink button and fallen petals, respectively, in 2015. The control reached lengths between 22.33 and 23.38 mm, and widths between 11.99 and 12.93 mm.
Conclusions: The use of the bioregulators showed significant favorable effects on dry weight, length and width of kernels at harvest, in both cultivars.

1. Introduction

The almond [*Prunus dulcis* (Miller) D.A. Webb] is a fruit species with a low chilling requirement, thus it begins flowering early in the season, coinciding with the end of winter, and consequently, the low temperatures, mists and rains typical in this period, which can have negative effects on pollination, fruit set and yield [1]. It is also important to consider that almond flowers are mostly self-incompatible and thus require cross-pollination [2]. For this reason, almond orchards should be planted with at least two intercompatible cultivars with simultaneous bloom, and will also require pollinators to transfer the pollen [3]. Pollination can be limiting in some areas of production; for example, in Chile, years with irregular spring weather conditions can have fruit set percentages of less than 20%, which has a direct impact on yields [4].

The unfavorable weather conditions during the period of almond flowering, in addition to the lack of adequate technology in managing the cultivars, can in some cases lead to very low yields in Chile, averaging 1400 kg ha^{-1} of kernel, compared with average almond yields in California that easily reach 2800 kg·ha^{-1} [5].

Pollen germination and fertility are affected by environmental factors such as light, temperature and relative humidity. The processes of seed and fruit development are intimately connected and synchronized, and are regulated by phytohormones [6]. Nevertheless, in contrast to the fruit, which can develop in the absence of pollination, seed development is strictly dependent upon successful fertilization. The development of the seed includes both the production of endosperm and the growth of the embryo, both of which have been shown to be under multihormonal regulation by auxins, cytokinins, gibberellins and brassinosteroids [7]. Successful fertilization is particularly important in the case of optimal yield for almonds, as the useful part of the fruit is the seed [8].

Cytokinins are a group of plant hormones that regulate cell division and influence numerous physiological processes of plant development, including: leaf senescence, vascular development, cell differentiation in apical meristems of shoots and roots, distribution and consumption of

* Corresponding author.
E-mail address: smmaita@uc.cl (S. Maita).
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nutrients, responses to biotic and abiotic stress, regulation of source–sink relationships, and recent studies have also revealed that cytokinin is a key regulator in seed production [9].

The principal physiological function of gibberellins in higher plants is the stimulation of organ growth by increasing cellular elongation and in some cases, cell division. In addition, gibberellins promote certain changes between seed dormancy and germination, juvenile and adult growth stages, and vegetative and reproductive development. In the performance of their functions, gibberellins act in response to both developmental and environmental signals [10]. Gallego-Giraldo et al. [11] also indicated that gibberellins are a key factor for fruit set and fruit development.

Brassinosteroids act on all parts of the plant, including the roots. These hormones provoke a wide range of physiological responses, including stem elongation, pollen tube growth, epinasty and leaf bending, inhibition of root growth, inducing ethylene synthesis, activating proton pumps, xylem differentiation, photosynthesis, synthesis of proteins and nucleic acids and activation of enzymes. In addition, brassinosteroids are also recognized as having a palliative role in plants that are subjected to abiotic or biotic stressors [12].

Phytohormones play a crucial role in modulating multiple developmental processes and cellular responses to stress of all kinds. Various natural plant hormones, such as gibberellins, salicylic acid, ethylene and brassinosteroids have been associated with cold stress responses in fruit [13].

Currently, the discovery and use of chemical substances that can replace or imitate the action of plant hormones, called plant bioregulators, has allowed growers to correct some deficiencies in order to prevent economic losses [14]. Plant bioregulators were rapidly identified as a means of improving yield, quality and post-harvest shelf-life, and have reached their greatest impact in the area of fruit production [15].

Swain et al. [16] suggested that gibberellins, presumably GA_1 and GA_3 synthesized in the embryo and/or endosperm, were required for the development of the seeds in the first days after fertilization. The application of gibberellins, and consequently, elevated levels of endogenous gibberellins, were causally associated with early growth of fruitlets and of mature fruit in *Pyrus pyrifolia* [17]. Gibberellic acid (GA_3), improved almond fruit set, and maximum fruit retention was observed with a concentration of 200 ppm [18]. Swain et al. [16] suggested two models for explaining the role of gibberellins in the distribution of assimilates; gibberellins could directly promote the consumption of assimilates for the development of seeds, or they could act indirectly through changes in the growth of seeds.

Exogenous application of synthetic cytokinins can induce fruit set and fruit development in various fruit crop species [19]. Zhao et al. [9], evaluated the effect of cytokinins in cotton, finding that a moderate concentration promoted the development of seeds, but an overdose inhibited their development. The application of adequate concentrations promoted seed development and increased seed size. Applications of kinetin, a synthetic cytokinin, in transgenic male-sterile tobacco plants resulted in normal fertilization and development of seeds [20].

In experiments with live plant cells, Vogler et al. [21] observed a five-fold increase in the rates of cell elongation when germination media were supplemented with 10 μM of epibrassinolide. Treatment of crops such as rice, tomato, corn and cucumber with brassinosteroids improved their resistance to low temperatures [12]. Alternatively, Thussagunpanit et al. [22] suggested that the application of brassinosteroids should be useful in increasing rice yields under high temperature conditions in the field.

This study evaluated the effects of three plant bioregulators (currently on the market) on the yields of Non Pareil and Carmel almonds, in an orchard in the area of Paine, in central Chile.

2. Materials and methods

This study was carried out during the 2013–2014 and 2014–2015 growing seasons in a commercial almond (*Prunus dulcis*) orchard located in the area of Paine, Metropolitan Region, in central Chile (latitude 33°46′21.3″ S – longitude 70°38′12.5″ W). This experiment evaluated the almond yields, in full production, from 10-year-old Non Pareil and Carmel cultivars grafted onto Nemaguard rootstocks, and planted at 6 m × 4 m. For the treatment applications, 12 trees of each cultivar were selected from alternating rows in a North–South direction.

2.1. Treatments

Three bioregulators that are currently on the market were applied to the trees to evaluate their effects on dry weight, length and width of kernels from Non Pareil and Carmel almond trees in the field:

a) Brassinolide 0.1%, wettable powder (WP), with the active ingredient 24-Epibrassinolide (chemical formula: 22R,23R,24R)-2α,3α,22,23-tetrahydroxy-24-methyl-β-homo-7-oxa-5-cholestan-6-one) made and marketed by Green Plantchem Company Limited, in the Republic of China.
b) ProGibb® 4%, soluble concentrate (SL), with the active ingredient Gibberellic Acid (GA_3) 3.2% *w/v* (chemical formula: 3S, 3aR, 4S, 4aS, 7S, 9aR, 9bR, 12S)-7, 12- dihydroxy-3-methyl-6-methylene-oxoperhydro-4, 7-methano-9b, 3-propenoazuleno (1,2-b) furan-4-carboxylic acid), made by Valent BioSciences Corporation, in the USA, and imported and distributed by Valen BioSciences Chile S.A.
c) X-Cyte®, soluble concentrate (SL), whose active ingredient is a cytokinin, kinetin, at a concentration of 0.04% *w/v* (chemical formula: 6-furfurylamino-9H-purine), made by Stoller Enterprises Inc. in the USA, and imported and distributed by Stoller Chile S.A.

These three plant bioregulators (PBRs): Brassinolide (BL), ProGibb® (GA_3) and X-Cyte® (KN) were sprayed at concentrations of 10, 30 and 50 $mg \cdot L^{-1}$, in the case of BL which is a wettable powder, and 10, 30 and 50 $\mu L \cdot L^{-1}$, in the case of GA_3 and KN, which are concentrated solutions. All of the concentrations used in this study correspond to commercially available products.

This experiment followed a random block design with 12 repetitions. For the treatment applications, 12 trees of each cultivar were selected, and then from each of these trees, 20 uniform branches from the middle of the canopy were selected. Ten of the branches were then selected at random to apply the treatments at the phenological stage of pink button, and 10 for treatment application at petal fall. The phenological stages were selected using the floral development scale for almonds proposed by Yi et al. [2], when 50% of the flowers were found to be at the proposed phenological stages used in this study [23].

Of the selected branches, treatments were assigned at random, including the control treatments, which consisted of spraying with water only. Each branch was considered an experimental unit.

2.2. Almond dry weight

When the almonds were found to be ripe, at least 80% open mesocarp (hull) [24], they were then harvested and brought to the Deciduous Fruit Crops Laboratory at the Campus of Agronomy and Forestry, of the Pontificia Universidad Católica de Chile. The fruit was then weighed (fresh weight), having been separated from the mesocarp and shell to obtain the almond seeds. Fresh weights were recorded for 10 kernels selected at random from each repetition (120 kernels per treatment). The kernels were then dried at 72°C for 24 h, until reaching a constant dry weight. After this process, the dry weight of the kernels was then recorded. All weighing was done using